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# ON THE ACTION OF CARBOLIC ACID UPON CILIATED CELLS AND WHITE BLOOD CELLS.

BY

**T. MITCHELL PRUDDEN, M. D.,**

*Lecturer on Normal Histology in the Medical Department of Yale College, Assistant in the Pathological Laboratory of the Alumni Association of the College of Physicians and Surgeons, New York.*



COMPLIMENTARY.

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# ON THE ACTION OF CARBOLIC ACID UPON CILIATED CELLS AND WHITE BLOOD CELLS.

By T. MITCHELL PRUDDEN, M.D.,

LECTURER ON NORMAL HISTOLOGY IN THE MEDICAL DEPARTMENT OF YALE COLLEGE, ASSISTANT IN THE PATHOLOGICAL LABORATORY OF THE ALUMNI ASSOCIATION OF THE COLLEGE OF PHYSICIANS AND SURGEONS, NEW YORK.

RICH as is the literature of the last two decades in records of investigations on the local and general action of carbolic acid upon the living organism, the minute changes which it produces in the cells themselves, seems to have attracted but little attention. To those who have adopted the postulate upon which the Listerian treatment of wounds is based, the study of the action of the antiseptic upon bacteria and allied organisms has seemed very naturally of paramount importance; while those, on the other hand, who denying, or at least not accepting, as yet proven, the theoretical considerations in which the Listerian treatment centres, but yet recognizing fully the value of carbolic acid as a topical application, seem to have contented themselves for the most part with very general explanations of its favourable action.

The significant fact that, other things being equal (sanitary conditions, care, etc.), the strict adherence to the Listerian doctrine and details, has, with perhaps the exception of certain classes of operations (ovariotomy, etc.), given results which, so far as statistical data are reliable, seem to be in no wise superior to those obtained by surgeons who use carbolic acid as a dressing agent, but without inflexible adherence to the details insisted upon by Lister, and which, if his postulate be true, would appear indispensable, has suggested the query, whether, after all, the beneficial effect of carbolic acid, which in some measure all acknowledge, may not really be largely due to its direct action upon the tissues of the healing wound. This view, which so far as it was formulated seems to have been the current one before the action of micro-organisms in wounds was postulated and became popular, was persistently urged by Beale[1] in 1872 on theoretical grounds, and has been ably advocated from the standpoint of the practical surgeon by Prof. T. M. Markoe,[2] in a recent number of this Journal.

[1] Beale, Disease Germs, Their Nature and Origin, 2d ed., 1872, p. 279, *et seq.*

[2] Markoe, Through Drainage in the Treatment of Open Wounds, Am. Journ. Med. Sci., April, 1880.

In the hope that some light might be thrown upon this important question by observations upon the action of carbolic acid on some of the elementary organisms of living tissues, a series of experiments was instituted the results of which it is the purpose of the present paper to record. The question proposed for solution was: What effects, if any, are produced by solutions of carbolic acid of varying strength, first upon the structure, and second upon the function of certain living cells? The two varieties of cells selected for this purpose were ciliated cells and white blood cells, and these were chosen because with a tolerably well-known structure one of the functional expressions of their life is in well-defined and easily studied movements.

The preparation used was Merck's purified carbolic acid, in crystals, and this was in all cases dissolved in a half per cent. solution of sodium chloride; the latter solution acting in most cases towards living tissues as an approximately indifferent fluid, or its effects being at least well known or determinate. Solutions were used of the strength of 1–20, 1–50, 1–100, 1–400, 1–800, 1–1600, 1–3200. The most extended series of experiments were made upon both ciliated and white blood cells from the frog (Rana esculenta), while less extended experiments were made upon both kinds of cells from the rabbit and upon the white blood cells of man. It does not seem desirable to record at length the individual experiments, but simply to describe the methods employed, and give their results. In each case a sufficient number of experiments was made to insure the elimination of such errors as isolated or a too limited number of observations in studies on the living tissues are always liable to lead, owing to varying conditions of vitality over which the investigator has no control, and of whose nature or presence he often may not even be aware. Control experiments, in which the indifferent fluid alone was used, were also made in all cases in which the character of the observation made them desirable.

*The Action of Carbolic Acid upon Ciliated Cells of the Frog.*—Its effect was first studied upon living cells scraped from the palate, and second upon the cells *in sitû* on the edge of the tongue of the living curarized animal, with the capillary circulation intact at their base.

In the first series of experiments, the cells having been gently scraped from the mucous membrane, were teased apart on a slide in half per cent. salt solution, protected from pressure by two bits of thin blotting paper on either side, and covered as usual by thin glass. After bringing into the field suitable masses of cells, in which the ciliary movement was vigorous, the carbolic acid in different degrees of dilution was allowed to flow under the cover-glass and was drawn off on the other side by blotting paper in the usual way. In order to avoid such errors as might arise from the concentration of the salt solution by evaporation, it is customary to inclose specimens exposed in this way to the action of fluids, with a rim of oil or paraffine; but when the observation is to be long continued, such an ex-

clusion of oxygen should be guarded against, and hence in these experiments a slow current of the irrigating fluids was kept constantly passing through beneath the cover-glass. It has been shown by Steinbuch, Purkinje and Valentin, Roth,[1] and others, that mechanical influences, such as currents of fluid, are capable under certain conditions of accelerating ciliary movement or even exciting it when it has once ceased. But a slow constant current being maintained in these experiments, alike with the indifferent and the carbolated fluid, this factor was probably made uniform for both.

Under these conditions the effects produced by carbolic acid are briefly as follows: In the strength of 1–20, the ciliary movement ceases on the instant of contact with the cell and the cilia loose their well-defined contours, shrinking to a tangled mass of curled and twisted filaments, which soon gives place to a narrow band of irregular fine granules. The cell body becomes at once deeply cloudy, and then coarsely granular, shrinking rapidly, and the protoplasm is soon resolved into two distinct kinds of material; the one clear, transparent, and feebly refractive: the other, formed in varying quantity, strongly refractive and forming irregular coarse shining reticular masses within the cell, or appearing in the form of larger and smaller shining globules or droplets, which may or may not entirely conceal the nucleus. Specimens kept under observation for 35 days showed within the first 48 hours some additional shrinkage, and a tendency to become brownish and dry in appearance, while the shining droplets usually became more or less coalescent and often freed themselves completely from the rest of the cell. After this time no marked change occurred in the cell within the above period. The nature of the shining globules and reticular masses developed from the protoplasm by carbolic acid, which are but partially soluble in alcohol and ether and not at all changed in colour by osmic acid or iodine, the writer was unable to discover.

The action of solutions of 1–50 and 1–100 are closely similar. In a small proportion of cases, especially when the ciliary movement is at all languid, it becomes distinctly accelerated as the acid reaches the cells, and then, which is true for most of the cells at first the movement becomes slower and usually ceases altogether in a few seconds, although in some cases, especially with the weaker solution, it may persist for several minutes. As the vibration ceases, the cilia may be indistinct in outline, or curved, or twisted, or may stand out from the cell straight and stiff. Then occur degenerative changes in the protoplasm similar to those above described, though less rapidly developed. Shining globules appear in the protoplasm, the nucleus shrinks, not unfrequently exhibiting as it does so

[1] Steinbuch, Analecten neuer Beobachtungen und Untersuchungen für die Naturkunde, 1802, p. 66. Purkinje and Valentin, De phenomeno motus vibratorii, etc. Wrastl., 1835. Roth, Virchow's Archiv, Bd. 37, p. 191.

distinct movements in the intra-nuclear network; and especially noteworthy in solutions of this strength, is the development in a large number of the cells, within a few minutes, of a wedge-shaped more or less strongly refractive, sharply outlined network, extending from the ciliary border to the nucleus, within which are sometimes seen delicate parallel lines, perpendicular to the free border of the cell. This peculiar network, lying invariably between the ciliary border and the nucleus and never extending below the latter, would suggest a difference in the structure of the protoplasm in this part of the cell which is not without significance in view of the present uncertainty of our knowledge concerning the relations between the cilia and the nucleus and cell body. Aside from a tendency of the shining globules to coalesce and gather outside the cell body within the first 48 hours, no marked change was noticed in cells exposed to the acid for 35 days.

Under the action of solutions of 1–200, the vibration is, at first, in many cases, distinctly accelerated and then becomes slower, the cell body becoming slightly cloudy, and ceases altogether as a rule within a few minutes. But it must be said that under these as well as other conditions, certain cells or groups of cells resist the action of the acid much longer than others which are exposed, so far as can be determined, to precisely the same conditions. After the movement ceases the wedge-shaped subciliary network as well as a few shining globules are sometimes slowly developed. But neither are as well marked or abundant as when solutions of greater concentration are used. Not infrequently, sometimes before the ciliary movement ceases, larger and smaller hyaline droplets may be seen exuding from the free border of the cell, often in great numbers.[1] If in the use of solutions of the above strength, at the instant when the vibrations cease, the carbolated fluid is washed out by the salt solution they may, under favourable circumstances, recommence and continue for a few minutes but cease permanently, as a rule, in a short time.

With solutions of from 1–400 to 1–800, the vibration is gradually brought to rest usually in from 60 to 90 m., the cilia standing out stiffly from the cell. The structural changes produced in the cell body are inconsiderable; they undergo the same sort of degeneration, but a little more slowly, observed in cells lying in simple salt solution. Here, too, if very soon after the cessation of movement the acid be washed out by salt solution, a restitution of the ciliary vibration often occurs, frequently continuing for a considerable time. It may even be again suppressed by the acid, and once more re-established by the indifferent fluid. In still more dilute solutions, 1–1600 to 1–3200, no characteristic structural changes

[1] This appearance, the occurrence of which under other conditions Virchow described many years ago (Virchow's *Archiv*, Bd. vi. p. 133), is instructive as showing how profound are the changes which cells may undergo without a suspension of functional activity.

were observed, but while exposed to the action of the acid, the movement was as a rule somewhat slower and came sooner to rest than when salt solution alone was employed.

A less extended series of observations upon the living ciliated cells from the air-passages of the rabbit showed that upon these the carbolic acid has essentially the same effect, but that they are somewhat more susceptible than those of the frog, both to influences causing structural changes and interference with functional movement.

In the second series of experiments on the ciliated epithelium, in which the action of the acid on the cells *in sitû* in the living animal was studied, the very convenient frog plate for the study of the tongue, devised by Prof. Richard Thoma, of Heidelberg, was employed.[1] If upon this apparatus the tongue be stretched as tightly as is compatible with the maintenance of a good circulation, and then covered and suitably irrigated, a most admirable view may be obtained at its edges of the ciliary movement under nearly normal conditions. Although the structural details of the cell bodies in this picture are not readily made out, the movements of the cilia are very distinct, and conclusions drawn from their study rest upon a much firmer basis than those derived from a study of the teased cells alone, since the latter, in the preparation for the experiment, are placed under excessively abnormal conditions. Still each method has its distinct advantages, and neither can be altogether dispensed with.

[1] This exceedingly useful apparatus consists of a rectangular plate of brass 12 cm. long by 7 cm. wide, covered above by a thin sheet of hard rubber. The plate is pierced at one end by an opening 2 cm. by 1 cm., into which a polished strip of glass is cemented which is thicker at the outer than at the inner end, corresponding to the difference in thickness of the frog's tongue at the tip and base. Around this glass plate over which the tongue of the curarized frog is gently stretched by pins driven into strips of cork on either side, a narrow rim of brass is fastened, making a gutter around the tongue from which two caoutchouc tubes lead off into a receptacle for the waste irrigating fluid under the table. On one side of the plate are two canula holders, so arranged as to turn in all directions, by means of which the irrigating fluids may be carried through fine glass tubes to any part of the tongue or made to irrigate the whole. The irrigating fluid is supplied from a Mariotte bottle set at a suitable height above the level of the microscope stage upon which the frog plate is clamped in the usual way, and the whole so inclined that the waste irrigating fluid will readily flow off.

The advantages of this apparatus are: that any surface exudation is washed away and the picture kept clean and clear; that the tongue may be kept for a very long time under observation, its proper moistening being automatical, and the waste fluids conveyed away without any danger of coming in contact with the microscope; that facilities are placed at the command of the investigator for varying in a multitude of ways the conditions under which the tissues are placed, the changes in irrigating fluids being effected simply by closing one clip and opening another. The tongue furthermore, notwithstanding its greater thickness at the base, is, owing to the obliquity of the glass plate upon which it rests, horizontally placed, and may be covered by thin glass, under which the fluids from the irrigating canulæ can be directed and studied with high powers. This plate is fully described and figured by Thoma in Virchow's *Archiv*, Bd. 65, p. 36.

This series of experiments, between 40 and 50 in number, showed that in general the action of carbolic acid upon the ciliated cells *in situ* is essentially the same as upon the separated cells, except that the former are considerably more resistant to its action. The same degenerative changes in the protoplasm are produced with the stronger solutions, and with these as well as with those considerably dilute a tendency to desquamation is usually observed. The most noteworthy effect produced upon the cells under these conditions by dilute solutions, is their power of holding the ciliary vibration distinctly in check without determining the death of the cell. It is thus possible with solutions of 1–800 to 1–1600 to keep the cilia moving at a very slow rate, but with quite perfect rhythm, for several hours, the movement being often first brought to a standstill by the desquamation of the cells. With solutions of from 1–400 to 1–1600, the movements may be brought to rest, and then, when desquamation has not occurred, restored by irrigation with the salt solution, and this may be done not once only, but four or five times. At each successive application of the acid, however, the inhibitory effect is more readily and quickly produced, until finally all vibrations cease abruptly on contact with the acid, and then the salt solution fails to restore it and the cells undergo the usual degenerative changes. The spectacle of the gradual re-establishment of movement on washing out the carbolic solution is extremely interesting. Sometimes it occurs in a steady progression from one side of the specimen to the other, the cilia at first vibrating at their tips through a very small arc, and then slowly swaying to and fro, and at last, sometimes almost instantly, breaking into a nearly normal rhythmic vibration, with the most perfect co-ordination of movement between individual cilia. Sometimes the re-establishment of vibration does not occur in a gradual uniform progression, but isolated cilia, or groups of cilia commence to vibrate independently of their neighbours, and may often be seen in full career while those about them are quite still. The same variations may be observed in the cessation of movement. After the cilia have been brought once or twice to rest by dilute solutions, the amplitude of their vibration in the subsequent restorations is, as a rule, much less than normal, their rate of vibration slower, and co-ordination of movement in neighbouring cilia or cell groups very imperfect. In a few cases it was noticed that at the instant of contact of the acid (1–400) with the cells, the cilia suddenly stood straight and stiff as if tetanized, and after remaining so for a few seconds as suddenly broke into rapid vibration, which gradually gave place to the slower movement, which is usual under the action of the agent.

The general results of the above experiments may be briefly enumerated as follows:—

Carbolic acid in moderately strong and concentrated solutions (1–100 and over) causes, either immediately or in a short time, cessation of vibra-

tion in living ciliated cells, with rapid characteristic disintegration of their protoplasm and death of the cell.

In very dilute solutions (1–400 to 1–3200) carbolic acid may cause, if long continued, slight degenerative changes in the protoplasm and the death of the cell; but its most noteworthy action is its inhibitory effect upon the ciliary movement. This may be retarded by it or even entirely checked without necessarily determining the death of the cell, for on its replacement by an indifferent fluid the movement may be perfectly re-established and continue indefinitely.

It is not necessary for the purposes of the present paper to enter into the very extensive literature of the ciliary movement, nor to consider at length in how far the above described effects of carbolic are similar to those produced by a number of other agents, the effects of which, ever since the classical investigations of Purkinje and Valentin, have been a favorite theme for amateur and professional study. Suffice it to say, that most of the agents which bring the vibrations to rest, do so, even in dilute solutions, in virtue of their destruction of the life of the cell. To these, sulphuric ether, chloroform, and carbonic acid are exceptions.

It should be further remarked that the power of certain dilute alkalies to temporarily restore ciliary movement, which has come to rest either spontaneously or by contact with certain fluids, is not analogous to that of the salt solution in the above experiments; for the latter acts by simply washing out a deleterious by an indifferent fluid, while the former induce profound structural changes in the cell, which speedily and invariably eventuate in death.

*The Action of Carbolic Action on Living White Blood Cells.*—These experiments again fall naturally into two series, in the first of which the effect of the acid was studied up in the cells taken from the vessels and exposed to its action upon the slide; while in the second its effects were observed upon the cells within the tissues and especially upon the phenomena of emigration. The method employed in the first series was essentially similar to that of the first series of experiments on ciliated cells, namely, irrigation beneath the cover-glass. Owing, however, to the liability of such cells as are fixed upon for observation to be floated out of sight by currents of irrigating fluid, great care in manipulation is necessary, and the following procedure was finally adopted as being the most uniformly successful. A small drop of one-half per cent. salt solution being placed in the middle of the slide, and the long middle toe of the hind foot of the frog[1] being carefully cleansed, its tip was snipped off with scissors, and the small drop of blood and lymph which presently exudes was carefully deposited in the centre of the drop of salt solution, and covered by

[1] It is very convenient to make use, for this purpose, of frogs, in which an artificial leucocythosis has been previously produced by snipping off the apex of the heart and allowing considerable blood to flow, and then closing the wound.

thin glass, the cells being protected by thin filter paper on either side. The advantage of depositing the blood in the drop of salt solution instead of directly upon the slide and mixing afterwards, becomes evident on examining the specimen, for under these circumstances the fibrine does not form in a close clot, but in a very loose network, frequently exhibiting a most exquisite radial arrangement of its fibrillæ about the fibrine granules. The loose network thus formed, while serving a most admirable purpose in preventing the floating off of the white blood cells, yet interferes but little with the free passage of the irrigating fluids in all directions. Irrigation was accomplished by allowing the fluid to drop at slow, regular intervals from a fine canula held by a clamp to the stage of the microscope and attached to a Mariotte pressure bottle; the overflow being drawn off at the opposite side by a strip of twisted filter paper.

If a specimen of frog's blood thus prepared, and irrigated with a half per cent. salt solution, be subjected to continuous observation, at a temperature favourable to the occurrence of amœboid movements, these may frequently be seen to continue for several hours, becoming after a time slower, and finally ceasing altogether, as the cells die, when, in a few hours they disintegrate, leaving but a few scattered granules behind. If, however, instead of irrigating with simple salt solution, a very dilute solution of carbolic acid in salt solution (1–800 to 1–1600) be used, the amœboid movements will be found not to continue as long and to be much more slowly executed, and to consist often, especially after the first half hour, not in locomotor movements, but simply in the extrusion and retraction of processes. And, when finally the cells cease to move, they do not disintegrate as rapidly as when the carbolic acid is absent. If the irrigating fluid is more strongly impregnated with carbolic acid containing from 1–800 to 1–400, the movements cease considerably earlier; while if 1–100 be employed, they cease almost as soon as the fluid reaches the cell, and very soon, if its action be continued, the protoplasm may be observed to resolve itself into larger and smaller strongly refractive granules or globules and a finely granular translucent substance, which preserves the form of the shrunken cell body, the nucleus becoming visible with more or less jagged contours. With solutions of 1–50 to 1–20 the movements cease instantly, the cells shrink, becoming coarsely granular, and very soon exhibit in most cases the above-described disintegration of the protoplasm.[1]

[1] The action of carbolic acid on the red blood cells has been very carefully studied and described in detail by Huels (Wirkung der Carbolsäure auf rothe Froschblutkörperchen-Inaug. Diss., Anklam, 1872). It will suffice here to say in general, that with dilute solutions they shrink, losing their regular contours and becoming variously folded and curved, and, after a time, may swell up, become pale, and disintegrate. With stronger solutions they become granular, and the protoplasm breaks up, forming a homogeneous, brownish-red, strongly refractive material, which may appear either as an irregular net-work within the cell or form globules, which tend to coalesce, and are insoluble in water, but are dissolved by carbolic acid.

Thus with very dilute solutions of carbolic acid the amœboid movement of white blood cells is held in check, but may still continue for several hours. With stronger solutions it either ceases instantly or very soon, and the protoplasm undergoes rapid decomposition.[1]

Such observations as the above are of course valuable only when made in each case with careful control experiments, in which the salt solution alone is employed, the other conditions remaining the same; and then definite conclusions are justifiable only as the result of a large number of observations; the individuality of the cells in their power of executing movements and in their capacity for resisting deleterious agencies, and the marked influence of the general well being of the animal from which they are taken, upon both, being always borne in mind.

Carbolic acid enjoys, and certainly deserves, the reputation of being, even in very dilute solutions, exceedingly inimical to the life of the lower organisms and to cell life in general. The question arises, however, at this stage of our investigation, are the cells whose movements have ceased through the agency of dilute carbolic acid, really dead? In the very numerous and valuable experiments upon its action on infusoria, bacteria, spermatozoa, etc., the cessation of movement seems to have been generally assumed to be the just criterion of death, and, inasmuch as the conditions of the experiment are usually so complicated and the organisms so minute, it is difficult to prove whether this is justifiable or not, because of the difficulty, in most cases the impossibility, of replacing the organisms under normal conditions so as to see whether or not their vitality is altogether lost or only held in abeyance. This, so far as disinfection is concerned, may, under certain circumstances, be of no little importance, and, in the determination of the capacities of protoplasm in general, it is certainly of the greatest significance. It has been shown in the first part of this paper that carbolic acid in dilute solutions is capable of bringing the ciliary movements to rest, and that if then it remains long in contact with the cells, they die and disintegrate, but if it is washed away in time, the movement may be re-established, showing that in the case of ciliated cells, cessation of movement does not necessarily imply the death of the cell. Is this true of the white blood cells, brought, as we have seen above, into a quiescent condition by dilute solutions of carbolic acid? If the carbolated fluid be carefully washed away from such quiescent cells, before it has been very long in contact with them, it will be found, in fact, that in a certain number of cases, cells which have been under continuous observation, will slowly commence to move again, at first sending out and retracting processes only, but at length exhibiting well-defined locomotion.

[1] Essentially the same results were obtained from a study of the action of the acid upon the white blood cells of the rabbit and of man. In this case the technique was modified by the use of the warm stage, the irrigating fluids being kept at a suitable temperature by the water-bath.

But the number of cells which do this is comparatively small. While such results are decisive, as determining that the cells brought to rest by means of dilute solutions of carbolic acid are not necessarily dead, it by no means follows that those which do not move by a substitution of the indifferent for the carbolated fluid *are* dead, for they are all, at best, placed under very abnormal conditions.[1] The following series of experiments will throw further light upon this subject.

*The action of Carbolic Acid on White Blood Cells within the Living Tissues.*—In these experiments the parts used were the tongue, the bladder, and the mesentery of the frog, and the results obtained were essentially the same for all.

For the study of the circulation in the bladder and mesentery, modifications of the above-described Thoma's frog plate were used, differing from that mainly in the size and shape of the plate upon which the animal rests, and the shape and position of the glass upon which the exposed part is laid for study. For the observation of the normal circulation and the phenomena of emigration the bladder is unsurpassed among all the parts of the frog which are used for this purpose. To prepare this organ for study, a lateral incision is made in the abdominal wall of a curarized animal, most conveniently on the right side, extending from the posterior end of the trunk about half way to the axilla, care being taken to avoid or ligate a large cutaneous vein which usually runs near the line of incision. A bent canula attached to a large pipette, filled with a half per cent. solution of salt is then introduced into the cloaca and directed forward into the bladder, and fastened in position with a bit of thread passed through the skin just above the anus. The salt solution now being injected into the bladder, either by the breath or by attachment to a suitable pressure flask, the organ as it fills is crowded out through the incision, and, being held in distension by a spring clip attached to the distal extremity of the pipette, it is laid upon the glass disk provided for that purpose in the frog plate and may be covered with thin glass and irrigated in the usual way. It can be examined with high powers, and leaves little to be desired in the clearness and precision with which both the normal and pathological conditions may be studied. It is, however, less well adapted for long continued observations of a special limited area or of a single cell, than the mesentery or tongue, because of the slow movement of the organ, due to the contraction of the smooth muscle cells so abundantly interlacing in its walls. This movement is more troublesome under ordinary circumstances than that induced in the mesentery by the intestinal contractions, because

[1] Special stress is laid upon this point, not because the capacity of reducing the activity of amœboid movements or bringing them to rest without killing the cells, is unique in carbolic acid, for we are already acquainted with many other substances which possess the same capacity, such as strong salt solutions, quinia, etc.; but for the sake of emphasizing in reference to cell activity, its inhibitory in distinction from its destructive power.

the bladder is exposed to the air over a greater area, and is less readily confined. It is worthy of note, however, that carbolic acid, even in solutions so dilute as to interfere but little with the circulation, very soon reduces this contractility of the smooth muscle cells of the bladder to such nearly complete inactivity, that the observation is but little interfered with. The methods of preparing the mesentery and tongue for study are so well known that nothing need be said here about the technique.

In all of these experiments, for obvious reasons, as small a quantity of curare was used at a dose as was consistent with the attainment of sufficient immobility of the animal.

If the freshly exposed bladder or mesentery of the frog, in which the circulation is active, be irrigated for an instant with a strong solution of carbolic acid (1–20) immediate stasis is produced in all but the larger vessels, the part becomes cloudy, and both white and red blood cells become rapidly shrunken and distorted, and degenerate in the manner above described, when the effect of the same solution was studied upon the extravasated cells. The bloodvessels change their calibre but little before stasis occurs in the larger trunks also, and the part very soon shrivels and commences to reveal the retrogressive metamorphoses which an entire absence of fresh blood involves. If these parts be irrigated with a more dilute solution (1–50 to 1–100) they soon become somewhat cloudy, and stasis will be seen to occur almost immediately in many of the capillaries (except those which form direct communication between larger arterial and venous trunks), gradually extending to the smaller and larger veins, and then to the arteries, which often dilate considerably, but without, as a rule, any perceptible acceleration of the current. One after another these vessels become crowded with red and white blood cells, the plasma becoming less and less in quantity, and usually within from twenty to ninety minutes all movement ceases, except in the largest trunks, in which a slow current may often continue for a considerable time longer. The details of the manner in which this stasis occurs may be best observed in the mesentery with solutions of 1–100, and with especial clearness in the smaller veins. One may observe in these, as the process advances along them from the direction of the capillaries, that the red blood cells no longer all present a symmetrical form, but are variously distorted, permanently curved or bent, sometimes appearing slightly swollen, while here and there one or more may be seen sticking closely with its side against the wall of the vessel. To this others may join themselves, forming a little heap of distorted cells encroaching upon the lumen of the tube, or, instead of being piled up in heaps, the red blood cells may lie along the wall, side by side, in a single or double layer, thus often forming a complete cylindrical investment to the inside of the vessel, through which the blood current may still pass with considerable, though markedly impaired, velocity. Gradually, however, more and more accumulation occurs until

the lumen of the vessel is finally entirely occluded, and the current stops. Then one may see in many cases how the cells become crowded closer and closer together until at last a uniform red mass fills the vessel, which soon becomes partially decolorized, and shows the nuclei with great distinctness. Sometimes the little collection of cells formed along the walls are loosened by the current, and driven along out of sight into the larger channels.

We have thus a most exquisite example of the formation of thrombi and emboli caused, partly at least, by evident changes in the red blood cells of which they largely consist. The white blood cells, under these circumstances, do not tend to collect in the peripheral zone of the current, which is very little developed, however long the circulation may persist; they invariably retain the spherical form, and are mingled indiscriminately with the red cells, and when the latter come to rest remain entangled among them, forming a small part of the thrombotic mass. This condition of affairs, already described by Hueter,[1] under the name of "globular stasis," although, according to that observer, readily undergoing resolution in the skin of the frog, where he chiefly studied it, does not do so when far advanced, even under the most favourable circumstances, in the bladder and mesentery. The appearance of the red blood cells as they lie against the walls, under these conditions, is not at all to be confounded with that often seen in diapedesis of the red blood cells. Where the latter process is occurring one or many cells may, indeed, be seen clinging to the wall of the vessel, but that part of the cell which is within, if it form a considerable portion of it, is pendulous, adhering at one point, and waves to and fro in the current, and is beaten about by passing cells. Nothing of this kind is seen in the cells changed by carbolic acid; they almost invariably lie flat against the wall, seem to be stiff and unyielding, and appear to have entirely lost their elasticity. That these effects are due to a purely local cause, is well shown by the fact that the distortion of the red cells and their adherence to the walls, and to one another, is first seen, and most evident throughout, either in the thinnest walled vessels, or in those in which the circulation is comparatively slow. Where fresh blood from the heart is being rapidly driven through the vessels, on the other hand, even though they be quite narrow and thin walled, as in some of the capillaries which form a short or direct communication between large arteries and veins, the changes are tardy in appearing.

If we turn now from solutions of carbolic acid of such concentration as to cause permanent stasis within a short time in these delicate parts, to those of such dilution that under their action the circulation may still con-

[1] Hueter, Algemeine Chirurgie, §§ 193 and 235; also Deutsche Zeitschrift für Chirurgie, Bd. 4, Heft 2, u. 3. This condition may, according to this writer, be produced by a number of agents besides carbolic acid, among which are alcohol, ether, chloroform, ammonia, glycerine, etc.

tinue active for several hours, namely, to solutions of the strength of from 1–800 to 1–3200, we find that within that range of dilution the effects produced are closely similar, and that when the parts are carefully prepared the circulation may often continue, with no tendency to stasis or thrombosis, for at least twenty-five hours, beyond which time observations were not extended. For the sake of convenience we may confine our attention to the effects produced by a solution of the strength of 1–1600, which may be regarded as typical. If we expose either the bladder or mesentery, and irrigate for a few moments with one-half per cent. salt solution, until the animal has recovered from the shock of the operation and the circulation has adapted itself to its new conditions, and then, cutting off the salt, irrigate with the carbolated solution of the above strength, we very soon observe a slight dilatation of both veins and arteries, with no marked change in the capillaries. This dilatation rarely exceeds one-eighth to one-quarter the original calibre of the vessel, and is permanent as long as the part is exposed to the carbolated fluid. The blood current becomes at the same time slightly accelerated for a few moments, and then usually becomes slower than normal, and so continues. At first the usual distribution of blood in the veins and arteries may be noticed, the axial current and the peripheral plasma zone, and within the latter, in the veins, a greater or less number of white blood cells may be seen rolling or gliding slowly along the walls, and even permanently adherent to them. Very soon, however, the plasma zone becomes less distinct, while the axial zone becomes somewhat broader, and by little and little a large proportion of the white blood cells which were at first fixed to the walls become loosened, and are carried off by the current. While still a considerable number are seen rolling along in the periphery, and even exhibit to a certain degree the well-known appearance of stickiness, this is very much less marked than when an indifferent fluid is used for irrigation, and very few cells, indeed, become permanently fixed. Those, moreover, which do drag along in the peripheral zone are, for the most part, globular, and are not drawn out into the well-known pyriform shapes presented under ordinary conditions. This condition persists as long as the carbolated fluid is continuously applied, and thus twenty-four hours and more of almost unremitting observation may pass, without the detection of a single cell in the act of passing through the walls, or moving in the tissues outside. Such experiments were often repeated, and while in no case was a cell actually seen passing through the wall, a few times, a small number of cells, having the characters of white blood cells, were seen to have appeared in the tissues outside the vessels, but in no case at a great distance from them. We are thus lead to the conclusion that, under the above conditions the irrigation of the mesentery or bladder of the frog with very dilute solutions of carbolic acid (1–1600) prevents, almost entirely, the emigration of white blood cells.

If now the carbolated fluid be replaced by one-half per cent. salt solution, the picture very soon changes. In the course of two or three hours, often much sooner in the bladder, without any marked change in the calibre of the vessels, the white blood cells begin to collect in the peripheral zone of the veins. They seem to be much more adherent to the walls as they glide along; they are seen to catch now and then at some point, and become elongated and drawn out into pyriform shapes, and very soon a greater or less number may be seen to be permanently fixed in the walls, through which certain of them are readily seen to slowly pass and wander off in the tissues. The process of emigration, then, may be held in check by dilute solutions of carbolic acid, but on the replacement of the latter by an indifferent fluid it readily occurs. If now, with emigration in full course, the carbolated fluid be again applied, a few cells may still be observed passing out of the vessels at first, but their movements soon become extremely slow, and shortly cease altogether; most of the white blood-cells in the peripheral zone gradually assume the spherical form and become less adherent, and the plasma zone is finally, to a considerable degree, freed from them.

Thus three times, in the same animal, in the course of twenty-four hours the alternation of irrigating fluids was accomplished, and each time with the same result.

These experiments may be varied in a number of ways. Thus the mesentery may be simply exposed to the air under irrigation with one-half per cent. salt solution, until emigration is actively progressing and the cells moving through the tissues and coming to the surface between the endothelium in the manner described in detail by J. Arnold,[1] when, on application of the carbolated fluid, not only does emigration soon cease, but also the locomotion in the tissues, and cells which were working their way slowly through on to the surface of the endothelial investment are brought to rest and so remain, often with a little shining knob through on the outside, while the remainder may be more dimly seen perfectly quiescent beneath. Cells which had been under observation in this condition for several hours were seen, on the replacement of the carbolated by the salt solution, to slowly drag themselves through on to the surface where they were floated off by the irrigating fluid. Still again, if a wound be made in the tongue by carefully dissecting off a bit of the mucous membrane from the papillary surface, emigration may be almost entirely prevented for many hours by irrigating with the dilute carbolated solution; or, if allowed to progress under an indifferent fluid, it may be suppressed at any time in the same way.

The most careful scrutiny failed to reveal any structural changes in the

[1] Arnold, Ueber die Durchtrittstellen der Wanderzellen durch entzündete Seröse Häute, Virch. Archiv, Bd. 74, p. 245.

white blood-cells rendered quiescent within the vessels or the tissues, by such dilute solutions as 1–1600.

These experiments were performed exclusively upon spring and summer frogs, which were for the most part freshly captured. Large or vigorous animals were invariably chosen, and, as above stated, a minimal dose of curare was always administered. Nevertheless it is not surprising, considering that the lethal dose for the frog is so small,[1] and that large quantities of the irrigating fluid were used to ensure uniformity in the conditions of the experiment, that in a certain small number of cases, when the irrigation was long-continued, a sufficient quantity was absorbed to cause death, or at least a considerable feebleness of the heart's action before the conclusion of the experiment.[2] All such cases were, of course, excluded in making the summary of the experiments on the action of the acid.

*Conclusions.*—The chief results of these investigations are as follows:—

Strong solutions of carbolic acid cause in living, active, ciliated, and white blood-cells, immediate cessation of movement and death of the cells, with speedy disintegration of the protoplasm.

Very dilute solutions of carbolic acid cause retardation or temporary cessation of movement in living, active, ciliated, and white blood cells, which may, under suitable conditions, be restored by the replacement of the carbolated by an indifferent fluid.

Strong solutions of carbolic acid, if applied to the bladder, tongue, or mesentery of the living frog, speedily cause stasis and thrombosis, partly, at least, through profound changes induced in the protoplasm of the red and, perhaps, the white blood cells.

Very dilute solutions of carbolic acid, if applied to the bladder, tongue, or mesentery of the living frog, under conditions in which inflammatory changes commonly occur, modifies the usual character of these, by preventing the occurrence of any considerable degree of emigration or locomotion of white blood cells in the tissues, the usual condition of affairs supervening, however, if the carbolated be replaced by an indifferent fluid.

One word should be added in conclusion, in reference to the relation of the above results to the process of emigration in general, and to the healing of wounds under the action of carbolic acid.

[1] Plugge, Pflüger's Arch., 1872, Bd. v. p. 540, estimates it as 0.010 grm.

[2] It must be confessed that this assumed relation between the feebleness of the heart's action and the absorption of the acid is, in large measure, conjectural; for, in addition to the incompleteness of our knowledge concerning the precise action of carbolic acid upon the heart under ordinary circumstances, the condition of the animal here, under the influence of curare, would doubtless render the exact condition of affairs still more obscure.

First. The more intimately we become acquainted with the process of emigration the less does it seem to possess the comparatively simple character with which it was at first credited. Neither the physical conditions determined by pressure, nor the relative or absolute position of the white and red blood cells within the vessels, nor the assumed glutinosity of the former, nor the existence of minute currents of fluid passing under certain conditions through the walls of the vessels into the lymph spaces outside, nor, finally, the locomotor capacities of the white blood cells themselves, seem alone to sufficiently explain this very curious and complex phenomenon. It seems probable that not the influence of one of them alone, but of all combined, and perhaps of others of whose existence we are not yet aware, is essential to its accomplishment. If this be true, we are at present by no means sufficiently acquainted with the action of the several factors to assign to each its appropriate share in the accomplished result. The latest supplement to the story of the investigation of the action of quinia on emigration[1] teaches, that it is by no means improbable that its inhibitory action is, in some cases, at least due to the change of physical conditions which it induces in the circulating blood, and not entirely to its power of restraining protoplasmatic movements.

Moderately concentrated solutions of salt also possess the power, as shown by Thoma,[2] of checking emigration, largely, probably, by the induction of a simple change in the physical conditions of the cells, and a dilatation of the bloodvessels and a consequent increase in the rapidity of the current. So in the above experiments, while the carbolic acid unquestionably exerts a paralyzing action upon the white blood cells, and seems to have little evident effect, so far as microscopical observation can determine, upon the bloodvessels, we can still only say with safety at present, that its action upon the white blood cells is at least one of the ways in which it holds emigration in check.

Finally, as to the relation of the results of these experiments to the use of carbolic acid in the treatment of wounds. One of the prominent claims to virtue in its action, both by the devotees to Listerism and the advocates of a modified use of the drug is, that it restrains undue suppuration.

Unfortunately the writer had not at his disposal the facilities for studying the action of carbolic acid upon the process of emigration in the warm-blooded animals, and the value of such observations upon the frog, as those described above, when applied to warm-blooded animals, and to man, each reader must finally decide for himself. The limitations, however, within which observations on one class of animals may be safely applied to another, are becoming daily more clearly defined, and it would seem to the

[1] Appert. Der Einfluss des Chinin auf die Auswanderung der weissen Blutkörper bei der Entzündung. Virch. Archiv, Bd. 71, p. 364.

[2] Thoma, Der Einfluss der Concentration des Blutes u. d. Gewebssäfte auf d. Form. u. Ortsveränderung farbloser Blutkörper. Virch. Archiv, Bd. 64, p. 1.

writer, at least highly probable, judging from the above experiments, that a part of the favourable action of carbolic acid in restraining undue suppuration in wounds is due to its direct effect upon the process of emigration, in so far at least as pus is the result of this process, and not of the proliferation of other cells. Whether beyond this it may or may not act beneficently in killing micro-organisms, or whether it may or may not have other less well-defined beneficial effects upon the healing process, are questions upon which these experiments throw no light.